REMARKS

Claims 1-11 were pending.

Claim 12 is new.

Claims 1, 2, 3, 4, 5 and 6 are amended.

Claims 1-12 are pending.

IDS

Applicants enclose the non patent literature missing from the file wrapper and entered on the September 1, 2006 IDS titled "Page 73, of Enzyme preparation and use, Chaplin & Bueke (1990), Cambridge University Press".

In regard to the foreign patent documents listed most have substantial US equivalents noted in the IDS mailed on September 1, 2006:

EP0707061	US5705382
EP0362829	US5089411
EP0243967	US4931391
EP0666321	US5563053
WO02/50297	US2004/048348

Inadvertently, the following equivalents were overlooked.

EP0307926	US5334519
WO90/12110	US5618687
SU1731814	No equivalent but enclose an English abstract

The Applicants apologize for the inconvenience.

Specification

The disclosure is objected to because the current address of the depository collection NCIMB is missing on page 11.

Applicants have amended the specification to add the specific address requested:

NCIMB Ltd.
Ferguson Building
Crabstone Estate
Bucksburn, Aberdeen
Scotland, AB219YA

35 USC 112, first paragraph

Please find enclosed herewith the deposit receipt and the viability statement for the strain NCIMB 41164 which describe all necessary items.

The material has been accepted for deposit under the Budapest treaty on the international Recognition of the Deposit of Microorganisms for the purpose of Patent Procedure and all restrictions on the availability to the public of the material so deposited will be irrevocably removed upon the granting of a patent.

35 USC 112, second paragraph

Claims 1-11 are rejected under 35 USC 112, second paragraph.

Claim **1** has been amended to clarify the meaning of "none actively growing free cells".

Basis for the amendment may be found on page 7, last paragraph.

Claim **1** is also amended to also define the term non-actively growing culture as the metabolism in the microorganism cells is substantially zero. Support for this amendment may be found on page 7, lines 27-28.

Claim **2** is amended to read:

A method according to claim 1 in which the microorganism is stored in step iii) as an aqueous paste comprising whole microbial cells.

Support for this amendment may be found on page 7, second paragraph.

Claim **3** is amended to read:

A method according to claim 1 in which the microorganism is stored in step iii) as an aqueous suspension of microbial cells in a suspending medium selected from the group consisting of water, physiological saline solution, a physiological buffer solution and an aqueous liquid containing at least one component of the growth medium.
Support for the above amendment may be found on page 7, paragraph 3.

Claim **4** is amended to read:
A method according to claim 1 in which the microorganism is stored in step iii) in the growth medium in step ii) .
Support for this amendment may be found on page 7, second paragraph.

Claim **5** is amended to delete the preferably phrase.

Claim **6** is amended to read:
A method according to claim 1 in which the growth medium comprised in the storage medium of step iii) further comprises urea or a urea derivative.
Support for this amendment may be found on page 10, lines 13-16.

No new matter has been added.

The applicants believe the above amendments correct the 112, second paragraph rejections.

35 USC 102(b)

Claims 1-5 and 7-9 are rejected under 35 USC 102(b) as being aniticpated by US 5,705,382, Endo.

Endo is directed to the stabilization of nitrile hydratase activity of microbial cells during storage. Endo does this by culturing the appropriate microorganism, collecting the resulting cells by centrifugation and washing, and then mixing the cell suspension with an aqueous solution of inorganic salts. The cells of Endo are preserved as a suspension or immobilized in an aqueous medium consisting of a neutral or weakly basic aqueous solution of inorganic salts having a molarity ranging from 100 mM to the saturation concentration of said inorganic salts. That is, Endo teaches the addition of a stabilizing agent for preservation of the cells. Additionally, the storage medium of Endo has no component of the fermentation broth .

The present claims have been amended to require the storage medium must comprise water and any residual fermentation broth components.

This amendment is based on page 8, lines 1-2 and page 12, lines 14-16. No new matter is added.

Endo teaches removal of the cells from the fermentation broth. This is quite clear in col. 3, lines 55-60 where Endo states:

> The resulting cells are collected by centrifugation and washed one or twice with a borate or phosphate buffer. Thereafter, the cell suspension is mixed with the aforementioned aqueous solution of inorganic salts in such an amount that the final concentration of interest is obtained.

The examples all confirm the absence of fermentation broth.

The present method requires the presence of residual fermentation broth (growth medium). As stated in the present disclosure in the first paragraph on page 9:

> "Storage stability may be achieved without resorting to removal of any of the fermentation broth components such as urea or urea derivatives."

Thus Endo does not anticipate the present claims.

In regard to claim 4:

Claim 4 should not have been included in the original anticipation rejection for the following reason.

Claim 4 (amended) reads:

A method according to claim 1 in which the microorganism is stored in step iii) in the growth medium in step ii) .

Endo removes the fermentation medium from the cells and does not make a single suggestion to do otherwise. Claim 4 requires that the microorganism is stored in step iii) in the growth medium in step ii). As Endo stores his cells only by first removing the fermentation medium, Endo cannot anticipate claim 4.

35 USC 103(a)

Claims 1-11 are rejected under 35 USC 103(a) as being unpatentable over US 5,705,382, Nagasawa and US 5,089,411.

US '382 is relied on as above. US '382 does not mention use of *Rhodococcus rhodochrous* NCIMB 41164. It does disclose another strain (co. 7, line 59) that is capable of producing acrylamide (col. 8, line 27, inventive example 6).

Both US '382 and US '411 teach the incorporation of urea in the culture media of *Rhodococcus rhodochrous.*

Therefore alleged by the examiner it would have been obvious to use *Rhodococus rhodochrous* cells stored as free cell suspensions and to use urea in the culture media.

Applicant agrees that incorporation of urea in the culture media is taught by US '382 and US '411. However, claim 6 is directed to the STORED microorganism in step iii) further comprises urea or a urea derivative.

Both US '382 and US'411 recommend adding urea or derivatives to the culture media only. US '382 teaches that once the cells are grown, the media is separated from the cells, thus the urea along with the other culture media or fermentation broth is no longer present with the stored microorganism. US'411 teaches only the use of urea and derivative in the culture media. US'411 makes no disclosure of a stored microorganism at all and thus make absolutely no suggestion that the stored microorganism further comprising the urea or derivative.

The present disclosure teaches that urea is a known protein denaturant, see page 2. ,line 25 to page 3, line 6: page 9, lines 5-7. Thus the prior art suggests removing urea before storing the microorganism.

Furthermore the combination of Endo with Nagasawa and US 5,089,411 does not arrive at the presently claimed invention. Endo does not suggest the presence of fermentation broth components within the storage medium, in fact teaches the removal of such components before storage. Neither of the secondary references make up for the definciencies of Endo. Thus the above rejection is inadequate as the combination of references does not complete the present claim limitations.

Reconsideration and withdrawal of the rejection of claims 1-11 is respectfully solicited in light of the remarks and amendments *supra*.

Since there are no other grounds of objection or rejection, passage of this application to issue with claims 1-12 is earnestly solicited.

Applicants submit that the present application is in condition for allowance. In the event that minor amendments will further prosecution, Applicants request that the examiner contact the undersigned representative.

Respectfully submitted,

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SAL\22350R1.doc

Shiela A. Loggins
Agent for Applicants
Reg. No. 56,221

Enclosures: Copy of English abstract for SU1731814, Copy of Page 73, of Enzyme Preparation and Use, Chaplin & Bueke (1990), Cambridge University Press and deposit receipt and the viability statement for the strain NCIMB 41164 which describe all necessary items

L2 ANSWER 1 OF 1 WPIDS COPYRIGHT 2008 THOMSON REUTERS on STN

AN 1993-141630 [17] WPIDS
DNC C1993-063677 [21]
TI Novel strain of Rhodococcus rhodochrous - is used as producer of nitrile hydratase
DC D16
IN ASTAUROVA O B; POLYAKOVA S V; YANENKO A S
PA (INMI-R) IND MICROORGANISMS GENETICS & SELECTION; (SAFI-R) SARAT SECT MICROORG SELECTION GENET PROM
CYC 1
PIA SU 1731814 A1 19920507 (199317)* RU 5[0]
ADT SU 1731814 A1 SU 1990-4826513 19900517
PRAI SU 1990-4826513 19900517
AB SU 1731814 A1 UPAB: 20050507

Novel Rhodococcus rhodochrous M8 VKPM S-926 strain is used as a producer of nitrile hydratase. The enzyme catalysts the hydrolysis of amides of carboxylic acids to amides. The strain 926 shows nitrile hydratase activity of 150 units, compared with 141 units for the known strain AM-324, and does not require the presence of expensive aminoacids in the nutrient medium.

Tests show that the activity of the ferment reaches 140, 220 and 150 mmoles/mg.min. w.r.t. isobutyronitrile, acetonitrile and acrylonitrie respectively.

USE/ADVANTAGE - In microbiology to grow new strain having high nitrile hydratase activity. Bul.17/7.5.92

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

CIBA Specialty Chemicals Water Treatments Ltd
P O Box 38
Cleckheaton Road
Low Moor
Bradford
BD12 0JZ

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page

EPO - DG 1

19. 07. 2006

NAME AND ADDRESS OF DEPOSITOR

76

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Rhodococcus rhodochrous BTR 2496	NCIMB 41164

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

[ ] a scientific description

[X] a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 5 March 2003 (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd.,

Address: 23 St Machar Drive,
Aberdeen,
AB24 3RY,
Scotland.

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorised official(s):

Terence Dodge

Date: 17 July 2003

[1] Where Rule 6/4(d) applies, such date is the date on which the status of International Depositary Authority was acquired.

Form BP/4 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

CIBA Specialty Chemicals Water Treatments Ltd
P O Box 38
Cleckheaton Road
Low Moor
Bradford
BD12 0JZ

NAME AND ADDRESS OF THE PARTY TO WHOM THE VIABILITY STATEMENT IS ISSUED

INTERNATIONAL FORM

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the INTERNATIONAL DEPOSITARY AUTHORITY identified on the following page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AS ABOVE Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 41164 Date of the deposit or of the transfer[1]: 5 March 2003
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 6 March 2003 [2]. On that date, the said microorganism was: [X] viable [ ] no longer viable	

[1] Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd.,

Address: 23 St Machar Drive,
Aberdeen,
AB24 3RY,
Scotland.

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorised official(s):

Date: 17 July 2003

[4] Fill in if the information has been requested and if the results of the test were negative.

Page 73, of Enzyme preparation and use, Chaplin & Bueke (1990), Cambridge University Press".

industrial use are sold on the basis of overall activity. Often a freshly supplied enzyme sample will have a higher activity than that stated by the manufacturer. This is done to ensure that the enzyme preparation has the guaranteed storage life. The manufacturer will usually recommend storage conditions and quote the expected rate of loss of activity under those conditions. It is of primary importance to the enzyme producer and customer that the enzymes retain their activity during storage and use. Some enzymes retain their activity under operational conditions for weeks or even months: most do not.

To achieve stability, the manufacturers use all the subtleties at their disposal. Formulation is an art and often the precise details of the methods used to stabilise enzyme preparations are kept secret or revealed to customers only under the cover of a confidentiality agreement. Sometimes it is only the formulation of an enzyme that gives a manufacturer the competitive edge over rival companies. It should be remembered that most industrial enzymes contain relatively little active enzyme (< 10% (w/w), including isoenzymes and associated enzyme activities), the rest being due to inactive protein, stabilisers, preservatives, salts and the diluent which allows standardisation between production batches of different specific activities.

The key to maintaining enzyme activity is maintenance of conformation, so preventing unfolding, aggregation and changes in the covalent structure. Three approaches are possible: (1) use of additives, (2) the controlled use of covalent modification, and (3) enzyme immobilisation (discussed further in Chapter 3).

In general, proteins are stabilised by increasing their concentration and the ionic strength of their environment. Neutral salts compete with proteins for water and bind to charged groups or dipoles. This may result in the interactions between an enzyme's hydrophobic areas being strengthened, causing the enzyme molecules to compress and making them more resistant to thermal unfolding reactions. Not all salts are equally effective in stabilising hydrophobic interactions, some are much more effective at their destabilisation by binding to them and disrupting the localised structure of water (the *chaotropic effect*, Table 2.4). From this it can be seen why ammonium sulphate and potassium hydrogen phosphate are powerful enzyme stabilisers, whereas sodium thiosulphate and calcium chloride destabilise enzymes. Many enzymes are specifically stabilised by low concentrations of cations which may or may not form part of the active site: for example, Ca^{2+} stabilises α-amylases and Co^{2+} stabilises glucose isomerases. At high concentrations (e.g. 20% (w/v) NaCl), salt discourages microbial growth due to its osmotic effect. In addition ions can offer some protection against oxidation to groups such as thiols by salting-out the dissolved oxygen from solution.

Low molecular weight polyols (e.g. glycerol, sorbitol and mannitol) are